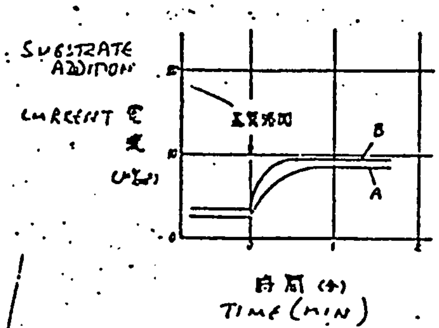
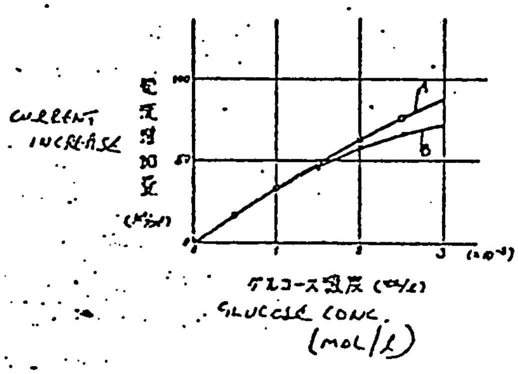
第4図



第5図



手続補正書

昭和54年2月22日

特許庁長官殿

1 事件の表示

昭和53年特許願第84481号

2 発明の名称

酵素電極およびその製造法

3 補正をする者

事件との関係　特許出願人

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5 補正の対象

明細書の発明の詳細な説明の欄

6 補正の内容

明細書第7頁第9行の「クロルアニル」を「ブロムアニル」と訂正します。

Japanese Patent Office

Patent ~~80~~ 65 - 10583 25/1/80

Application 78 - 84481 10/7/78

Specification

1. Name of Invention

Enzyme electrode and its method of production

2. Scope of Claim

1) An enzyme electrode characterised in that it has a layer 1 consisting of an electron conductive substance, a layer 2 consisting of the electron conductive substance and an insoluble redox compound, and a layer 3 containing a fixed enzyme and moulded on the above recorded layer 2.

2) An enzyme electrode as in 1) of the claim where layer 3 contains the above enzyme and its co-enzyme.

3) A method of production of an enzyme electrode characterised in that a layer 1 consisting of an electron conductive material and a layer 2 consisting of a mixture of an insoluble redox compound and the electron conductive material are moulded into a body, and then a layer containing an immobilized enzyme is applied on to this above-recorded layer 2.

3. Detailed Specification

This invention is intended to provide an enzyme electrode possessing electrochemical activity against the substrate on which the specific catalytic action of the enzyme operates, capable of rapid and simple measurement of the concentration of the substrate, and capable of continuous and repeated use. This invention also relates to an enzyme electrode which, by its combination with an oxygen electrode, may be used in a cell to convert the chemical energy of the substrate into electrical energy.

As an example of an attempt at an engineering application of the specific catalytic action of enzymes, trials using an enzyme reaction system, combined with an electrochemical reaction system, have been carried out on the detection of concentration of a substrate which is the substance that reacts specifically with the enzyme. In treating an enzyme reaction as an electrochemical reaction, the method has been employed, for example, where a suitable redox compound has

been introduced conjugately into an enzyme reaction system, and the oxidation-reduction reaction of this redox compound has been detected electrochemically. More concretely, the redox compound which has been reduced (or oxidised) in the conjugate reaction with the enzyme is oxidised (reduced) electrochemically, and the substrate concentration at this time can be detected as a current flow.

However, because the expensive enzyme and redox compounds are used in the dissolved state, they are lost each time a measurement is made, and the operation of the measurement itself is troublesome. In order to resolve these difficulties and facilitate repeated use of the enzyme, etc. and also produce a practicable enzyme electrode, it is necessary that the enzyme and redox compound be fixed into one body with an electron conductive substance as a collector.

As the result of various tests on methods of obtaining enzyme electrodes, where an enzyme and a redox compound are fixed in one body, the method has been discovered of using carbon powder, for example, as the electron conductive material, press-moulding a mixture of this with an insoluble redox compound, and immobilizing the enzyme on the moulding. Another method is to mix carbon powder on to which the enzyme has previously been fixed into the above-recorded mixture. Enzyme electrodes thus obtained are capable of rapid and simple measurement of a substrate concentration.

As an improvement of the enzyme electrode, this invention, by virtue of its being constructed using a layer 1 consisting of an electron conductive substance, a layer 2 consisting of the electron conductive substance and an insoluble redox compound, and a layer 3 containing an immobilized enzyme, has successfully produced an enzyme electrode of high performance which greatly reduces the amounts of the enzyme and redox compound used.

Fig. 1 shows an example of the construction of an enzyme electrode according to this invention. In this Figure, 1 is the layer consisting of the electron conductive material, 2 is the layer consisting of the insoluble redox compound and the electron conductive substance, and 3 is the layer containing the immobilized enzyme, the insoluble redox compound being conjugate with the above-mentioned enzyme. These three layers are formed by moulding into one body.

Layers 2 and 3 are the parts which bring about the reaction between the substrate and the enzyme + redox compound, while layer 1 functions as the base

and collector for layers 2 and 3. As Fig. 2 shows, this 3 layer construction of reaction layers may be formed on both sides, as necessary.

Thus it is possible to construct an electrode with the minimum necessary amounts of enzyme and insoluble redox compound.

Next a method of measurement using an enzyme electrode will be described.

Fig. 3 shows a measurement system for the measurement of concentration of a substrate using an enzyme electrode according to this invention. In the Figure, 4 is the recorder, 5 is the potentiostat, 6 is the reference electrode, 7 is the salt bridge, 8 is the opposite electrode, 9 is the electrode holder to which is fitted the above-recorded enzyme electrode, 10, and 11 is a phosphoric acid buffer solution of pH 5.6 containing the substrate.

The enzyme electrode 10 is attached to the holder 9 so that the layer 3 containing the immobilized enzyme is in contact with the buffer solution 11; and to layer 1 of the electron conductive substance is attached a lead of, for example, platinum. After the enzyme electrode has been immersed, and after the electrode potential has been kept at a fixed potential agaist the reference electrode, the amount of variation in the oxidation-reduction current of the redox compound which is varying the substrate concentration is detected. It may be said that the greater the current variation per unit concentration of the substrate, or the wider the range of substrate concentration over which a linear relationship holds between the substrate concentration and the current variation, the better the electrode performance.

In the case of the enzyme electrode of this invention, the immobilized enzyme layer comes into contact with the specimen solution, the substrate diffuses into the enzyme layer, penetrating into the electrode, and reaction commences. Thus, as the transfer of the substrate is controlled by the enzyme layer, the previously described linear relationship holds even with comparatively high substrate concentrations. As in this enzyme electrode the optimum construction of small amounts of enzyme and redox compound with the electron conductive material is obtained, it achieves effective utilization of these expensive enzymes and redox compounds.

Metals which are stable in oxidation-reduction, such as carbon or tin oxides and other such conductive metallic oxides, may be used as the electron conductive substance. In particular, carbon is a stable substance with good

conductivity; also it does not obstruct the enzyme reaction, and is a favourable electron conducting material.

Now follows a description of the method of production of this enzyme electrode.

First the electron conducting substance and insoluble redox compound, reduced to powder, are well mixed. Next a small amount of this mixture and the electron conductive substance are moulded into one body by, for example, press-moulding. A suitable binder may be used to increase the strength of the moulding. In the moulding thus constructed from two layers--one consisting of an electron conductive material, and a second consisting of the electron conductive material and an insoluble redox compound--the enzyme, and if necessary the co-enzyme also, is immobilized on to the layer containing the insoluble redox compound. Various methods may be employed for the immobilization of the enzyme, such as the method using a cross-linking reagent such as glutaldehyde.

Below is given a description of this invention by means of an embodiment. Carbon powder such as acetylene black, graphite, etc. as the electron conductive material, and bromanil as the insoluble redox compound are well mixed. Then a small amount of this mixture and some carbon powder are press-moulded into one body. On to the layer containing the 'chloranil' (sic) thus obtained, the oxidising-reducing enzyme glucose oxidase is fixed by means of glutaldehyde. This is enzyme electrode A according to this invention.

As an enzyme electrode for the purposes of comparison, carbon powder on to which glucose oxidase had already been fixed and bromanil were well mixed and then press-moulded. This is enzyme electrode B.

Fig. 4 shows the variations in current values when the above-mentioned enzyme electrodes were used with a glucose concentration of 2×10^{-4} mol/litre. Fig. 5 shows the relationship between glucose concentration and current increase. As is clear from the figures, enzyme electrode A, like enzyme electrode B which has an immobilized enzyme layer, responds rapidly to the addition of the substrate, and has excellent characteristics, such as an increase in response linearity against rises in substrate concentration, and so on.

Where a co-enzyme such as alcohol dehydrogenase is necessary, when the co-enzyme is fixed together with the enzyme, favourable response characteristics similar to those above are obtained. As well as bromanil, the redox compound

may be any insoluble redox compound such as chloranil or various types of redox polymers etc.

As described above, by means of this invention, an enzyme electrode may be obtained simply which has excellent characteristics and is designed for effective use of enzyme and redox compound.

4. Simple Description of Figures

Fig. 1 shows an example of the construction of an enzyme electrode of this invention; Fig. 2 shows another example of construction; Fig. 3 shows a system for the measurement of substrate concentration; Fig. 4 shows the response characteristics of the enzyme electrodes against glucose; and Fig. 5 shows the relationship between glucose concentration and current increase.

1 . . . . . . layer 1

2 . . . . . . layer 2

3 . . . . . . layer 3

Name of Agent, Nakao Toshio, Attorney-at-law and one other.

Procedural Amendment 22/2/79

1. re Application 78/84481
2. Invention title:

   Enzyme Electrode and Its Method of Production
3. Appellant Yamashita
4. Agent Nakao
5. Section on Detail Specification
6. Page 7 line 9 of original document, for 'chloranil' read 'bromanil.'